

# Effects of Nursery Pre-Conditioning on *Panicum hemitomon* and *Sagittaria lancifolia* Used for Wetland Restoration

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## Abstract

A study was conducted to examine nursery protocols for production of planting stocks used in wetland mitigation projects. Two commercial soil mixtures were tested along with waterlogging, fertilization, and combination treatments. Two marsh species, *Panicum hemitomon* and *Sagittaria lancifolia*, were subjected to a two-phase study. During Phase I, watering and fertilization treatments were applied in a  $2 \times 2 \times 2$  factorial design with two soils, two watering regimes, and two fertilizer treatments. In Phase II, all plants were subjected to continuous waterlogging (no fertilizer). Soil redox potential was measured, along with plant gas exchange and growth responses. Our data do not support the hypothesis that flood "pre-conditioning" alone can significantly improve plant growth under subsequent flooding. However, fertilization alone or in combination with flooding appeared to enhance shoot and root production in both species during the subsequent flooding. In contrast, flooding alone produced *Panicum* plants that appeared to remain somewhat susceptible to subsequent flooding as compared to fertilized

plants. *Sagittaria* plants subjected to fertilizer treatment alone did not produce significantly greater total dry weights compared to their controls. Our data indicate that the growth of planting stocks for wetland mitigation can be improved by fertilization in the nursery.

**Key words:** fertilizer, flooding, nursery production, wetland mitigation.

## Introduction

The rapid loss of wetland resources in many parts of the United States has received much attention in recent decades and measures are being pursued by various agencies to prevent and/or mitigate such losses (Mitsch & Gosselink 1993). Thus, the need for commercial production of planting stocks is greater than ever. Information is needed for effective adoption of horticultural nursery practices for marsh nurseries to produce stocks for marsh mitigation. However, such information at present is limited (McIninch et al. 1997). If stocks are grown in the nursery using the existing conventional horticultural protocols, they are exposed to optimum conditions of soil moisture and nutrients, unlike the stressful conditions that they may face upon field transplanting at mitigation sites. Such planting stocks are likely to be vulnerable to the stressful field conditions on a wetland site that may be characterized by flooding, intensely reduced soil conditions (low soil redox potential,  $E_H$ ), and soil nutrient imbalance. Slight modification in nursery practices by growing "pre-conditioned" plants using proper rooting medium, exposing plants to short-term flooding, and fertilizer application may pre-condition plants for actual field situations and, thus, ultimately enhance marsh mitigation efforts. Literature suggests that proper nutrition management in the nursery enhances field performance of transplanted stocks (Lee et al. 1992; Compton & Nelson 1997). Fertilization during seedling production in the nursery improved the subsequent field performance of several vegetables (Tremblay et al. 1987) and grasses (Pitman & Read 1998). Seedlings of several species grown in plugs produced greater dry mass upon transplanting than non-fertilized seedlings (Van Iersel et al. 1998). However, the effects of flood pre-conditioning are less clear. Studies so far have indicated that flood pre-conditioning of containerized seedlings of woody species did not improve field survival under permanently or temporarily saturated soils (McIninch et al. 1994).

The present study was designed to test the hypothesis that certain nursery pre-conditioning may aid in producing pre-conditioned planting stocks that grow better under stressful wetland soil conditions than non-pre-conditioned stocks. We proposed the following questions: To what degree can plant survival and growth be enhanced through flood pre-conditioning

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(i.e., application of flood cycles on seedlings in the nursery)? How reduced does the commercial potting soil become upon flooding and would such  $E_H$  represent the level of  $E_H$  found in a typical wetland ecosystem? Do fertilized plants have a better survival rate than non-fertilized plants upon transplanting? Is there any interaction between flooding and fertilization leading to the production of vigorous stress-tolerant planting stocks? The objective of the study was to test the effects of different commercial soil mixtures, short periods of flood pre-conditioning, and fertilization during the early stages of plant development in the nursery on subsequent physiological functioning, survival, and biomass productivity under flooded conditions typical of those expected in the field for many wetland mitigation sites.

### Materials and Methods

Seedlings of two freshwater marsh species used in wetland mitigation projects in the south-central United States (Mitsch & Gosselink 1993), *Panicum hemitomon* Schult. (maidencane) and *Sagittaria lancifolia* L. (bull-tongue), were used in this study. The plants and their associated roots were collected in the winter of 1997 and transferred to a climate-controlled laboratory. Plant propagules then were transplanted into propagation flats 50 × 26 × 6 cm. The newly regenerated plants were then separated using a method described by Pezeshki and DeLaune (1993), and transferred from propagation flats into pots 15 cm in diameter and 20 cm deep. The pots contained one of the following two commercial soil mixtures: (1) Metro Mix or (2) Jiffy Mix. Plants were then monitored closely for a week and any dead plants were replaced.

The experiment was divided into two phases to evaluate the effects of soil saturation (waterlogging) and fertilization during Phase I (simulated nursery conditions) on the subsequent performance of plants under continuously waterlogged conditions in Phase II (simulated post-transplanting period in the field). In Phase I, watering and fertilization treatments were applied to each soil type according to the following protocol: (1) waterlogging and fertilizer; (2) waterlogging, no fertilizer; (3) no waterlogging, fertilizer; and (4) no waterlogging, no fertilizer. The treatment combinations and abbreviations are shown in Table 1. The no waterlogging, no fertilizer (NWF-) was used as the control because it mimics conditions that exclude the treatments being tested. Watering treatments were: (1) non-waterlogged (watered daily but allowed to drain to field capacity and, thus, not flooded); and (2) waterlogged (water levels maintained at 5 cm above the soil surface, thus, maintaining soil in saturated status). Plants in the fertilizer treatment in Phase I were fertilized weekly with

100 ml of 20-20-20 Peters fertilizer mixed at a concentration of 1.25 grams per liter. Phase I treatments continued for a period of 42 days. Ten *Panicum* and four *Sagittaria* plants were randomly assigned to each treatment combination, for a total of 80 and 32 plants, respectively. At the end of Phase I, all flooded plants were drained for 3 days before Phase II began. At this time, four *Panicum* plants per treatment were destructively sampled. No *Sagittaria* plants were destructively sampled owing to the limited number of plants for this species. In Phase II, all plants remained in the laboratory and were subjected to continuous waterlogging (no fertilizer) to test the effects of pre-conditioning imposed in Phase I. Phase II was conducted for a period of 38 days. Sample sizes in Phase II were six and four pots per treatment for *Panicum* and *Sagittaria*, respectively. Throughout the study, plants were grown under laboratory conditions of 16 hours light and 8 hours dark, with temperatures of  $26.0 \pm 0.5$  and  $22.0 \pm 1.0^\circ\text{C}$ , respectively. The light at canopy level,  $1,000 \pm 120 \mu\text{mole/m}^2\text{sec}$ , was provided by a multi-light water-cooled system consisting of high-intensity lamps (Sun-Brella, Environmental Growth Chamber Inc., Chagrin, Ohio).

The experiment followed a  $2 \times 2 \times 2$  factorial design with two levels of soil types that are commercially available (Jiffy Mix, Metro Mix), two watering regimes (waterlogged, drained), and two fertilization regimes (fertilized, not fertilized). Treatments were randomly blocked to control any potential variation in environmental conditions in the laboratory.

### Soil Measurements

Soil redox potential ( $E_H$ ) was measured once weekly at 15 cm from the surface to quantify reduction status in flooded and non-flooded treatments during both phases.  $E_H$  in the potting soil was measured using platinum-tipped redox electrodes, a calomel reference electrode, and a millivoltmeter (Orion Model 250A).

**Table 1.** List of treatments imposed during Phase I including soil type, watering regimes (W = waterlogged, NW = no waterlogging), and fertilizer treatment (+ = fertilized, - / not fertilized).

Soil Type	Watering Regime	Fertilizer Treatment	Abbreviation
Jiffy Mix	NW	+	JNWF+
Jiffy Mix	NW	-	JNWF-
Metro Mix	NW	+	MNWF+
Metro Mix	NW	-	MNWF-
Jiffy Mix	W	+	JWF+
Jiffy Mix	W	-	JWF-
Metro Mix	W	+	MWF+
Metro Mix	W	-	MWF-

## Plant Gas Exchange Responses

Measurements of photosynthetic photon flux (PPFD), stomatal conductance (SC), and net photosynthesis (NP) were conducted using a portable photosynthesis system (Model CIRAS1, PP Systems Inc., Haverhill, MA). Gas exchange measurements were conducted on five *Panicum* and four *Sagittaria* leaves per treatment (one leaf per each sample plant) in the upper third of the plant canopy on each sample day. Measurements were conducted under steady light levels in the laboratory. Measurements were conducted on four sample days in Phase I (day 7, 14, 28, and 35) and three sample days during Phase II (day 50, 64, and 77). Leaf tissue chlorophyll concentration was measured according to the method described by Hiscox and Israelstam (1979). Four leaf samples from each species-treatment combination were measured at the conclusion of the study to quantify chlorophyll concentrations. Leaf samples weighing between 0.221 and 0.334 g were cut, placed in 10 ml of Dimethyl Sulfoxide (DMSO), and incubated at 70°C for two hours. A spectrophotometer (Turner, Model 690, Dubuque, IA) was used to determine chlorophyll concentrations.

## Plant Growth Responses

Height and number of culms in each pot were measured weekly during Phase I. The same variables were measured once every 2 weeks during Phase II. *Panicum* was the only species that produced culms. At the beginning of the study, initial biomass for plant samples of each species was estimated by sampling twelve and four destructive sample plants from *Panicum* and *Sagittaria*, respectively. Biomass of all study plants was sampled at the conclusion of Phase II. Biomass samples were divided into shoot and root components and dried at 75°C until they reached a constant weight. Root porosity, a measure of the percentage of air space within a root, was measured on the five *Panicum* samples used for biomass analysis at the end of Phase I and on all plants of both species at the conclusion of the study. Root porosity was measured using the pycnometer technique as described by Jensen et al. (1969).

## Data Analysis

The general linear models (GLM) procedure of the Statistical Analysis System (SAS Institute 1990) was used to test differences in means among treatments within species and *t*-tests were used to test for differences between species within each treatment for growth, biomass, root porosity, and chlorophyll concentration. Repeated measures analysis (Moser et al. 1990) was used to test for significant differences in gas exchange response parameters and interactions.

## Results

### Phase I

Flooding produced similar results in both commercial potting soils during Phase I of the study. Soil  $E_H$  in the non-waterlogged treatments remained above +450 mV, indicating well-aerated conditions (Fig. 1). In waterlogged pots, soil  $E_H$  decreased shortly after treatment initiation and remained reduced during the remainder of Phase I (Fig. 1).

**Fertilization (MNWF+ and JNWF+).** Mean stomatal conductance (SC) and net photosynthesis (NP) measured during sample days of Phase I for fertilized *P. hemitomon* were not significantly changed compared to their respective controls (JNWF-, MNWF-) (Table 2; Fig. 2A). Similarly, no significant difference in height growth, number of culms, root biomass, total biomass, and root porosity was found in fertilized *Panicum* compared to controls (Table 2). By the end of Phase I, shoot biomass in fertilized Jiffy Mix soil was increased significantly, indicating that fertilization resulted in increased above-ground biomass (Table 2). Leaf tissue chlorophyll concentration was enhanced significantly in fertilized Metro Mix soil compared to controls (MNWF-) (Table 2). In *S. lancifolia*, SC and NP were significantly higher in fertilized plants grown in Jiffy Mix soil as compared to controls (JNWF-) (Table 2; Fig. 2B). Height growth in *Sag-*

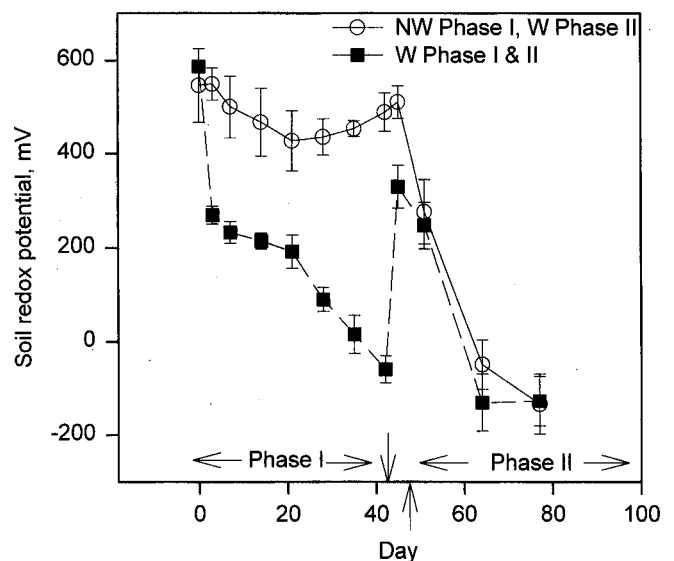


Figure 1. Mean weekly soil redox potential ( $E_H$ , mV) during the study. Non-waterlogged pots (NW) were well watered and well drained during Phase I of the study. Waterlogged pots (W) were flooded to 5 cm above the soil surface during Phase I. During Phase II, all pots were flooded to 5 cm above the soil surface. Bars represent the mean  $\pm$  SE.

*ittaria* was not significantly affected compared to the respective control (MNWF–, JNWF–) (Table 2).

**Waterlogging (MWF– and JWF–).** Mean SC and NP of waterlogged *Panicum* grown in Metro Mix were significantly decreased compared to controls (MNWF–), whereas no significant difference for the same variables was found in plants grown in Jiffy Mix (Table 2; Fig. 2A). Dry root biomass and total biomass were significantly reduced in Jiffy Mix in response to soil flooding, whereas no difference was found between waterlogged *Panicum* plants grown in Metro Mix (Table 2). No significant differences in height growth, number of culms, and root porosity were found in waterlogged *Panicum* compared to controls (Table 2). Further analysis of root porosity data was performed to test the effect of soil flooding. Treatments were split into plants that were flooded and non-flooded, and analyzed using a *t*-test. Root porosity of flooded plants (mean = 31.3%, SE = 2.8) was significantly greater ( $p = 0.004$ ) than non-flooded plants (mean = 19.0%, SE = 2.5), indicating flood-induced enhancement of aerenchyma tissue development in *Panicum*. In *Sagittaria*, SC of waterlogged plants grown in Jiffy Mix was significantly increased compared to controls (JNWF–), whereas NP of plants grown in Metro Mix under the same conditions was significantly decreased compared to controls (MNWF–) (Table 2; Fig. 2B). Height growth of *Sagittaria* was not significantly affected by waterlogging (Table 2).

**Fertilization and Waterlogging (MWF+ and JWF+).** The number of new culms formed on fertilized and waterlogged *Panicum* plants over the course of Phase I was signifi-

cantly increased in both soils compared to the respective controls (JNWF–, MNWF–) (Table 2). Other variables such as SC, NP, height, shoot and root biomass, leaf tissue chlorophyll concentration, and root porosity were not significantly affected by the combined fertilization and waterlogging in either soil treatment (Table 2; Fig. 2A). In *Sagittaria* grown in Jiffy Mix, mean SC and NP were significantly increased by the combined fertilization and waterlogging compared to controls (JNWF–) (Table 2; Fig. 2B). Mean NP of *Sagittaria* grown in Metro Mix was significantly decreased compared to controls (MNWF–) (Table 2B).

Analysis of variance (ANOVA) results revealed significant interactions between flooding and fertilizer ( $p = 0.018$ ) and among soil, flooding, and fertilizer for SC ( $p = 0.014$ ). There were also significant interactions between flooding and fertilizer ( $p = 0.025$ ); species, soil, and fertilizer ( $p = 0.015$ ); and species, flooding, and fertilizer for NP ( $p = 0.014$ ). The ANOVA results reflected the difference in gas exchange responses of the two species to various treatment combinations.

## Phase II

At the initiation and during Phase II, which began on day 45 of the experiment, all plants were subjected to waterlogging only (no fertilizer). Symbols used hereafter refer to treatments during Phase I of the study. Soil  $E_H$  in pots dropped into the range associated with reducing soil conditions (Fig. 1). Soil  $E_H$  in all pots became reduced as Phase II continued, regardless of the pre-treatment watering regime; at the conclusion of Phase II,  $E_H$  ranged between –128 and –134 mV (Fig. 1).

**Table 2.** Responses of height growth (final height – initial height, cm), number of culms per pot (final number – initial), shoot dry weight per pot (g), root dry weight per pot (g), total dry weight per pot (g), leaf tissue chlorophyll concentration (mg/g FW), stomatal conductance (SC, mmol H<sub>2</sub>O/m<sup>2</sup> leaf area/s), and root porosity (% airspace) for *Panicum hemitomon* during Phase I; also presented are height growth (final height – initial height, cm), and stomatal conductance (SC), for *Sagittaria lancifolia* during Phase I.\*

	Treatment							
	JNWF+	JNWF–	JWF+	JWF–	MNWF+	MNWF–	MWF+	MWF–
Species	<i>Panicum hemitomon</i>							
Height growth	48.9 (2.9) <i>a</i>	39.2 (2.6) <i>ab</i>	41.1 (1.0) <i>ab</i>	36.2 (6.7) <i>b</i>	36.6 (6.8) <i>a</i>	38.7 (5.3) <i>a</i>	36.4 (6.6) <i>a</i>	34.4 (6.0) <i>a</i>
Number of culms	4.7 (0.7) <i>b</i>	4.3 (0.6) <i>b</i>	10.7 (1.3) <i>a</i>	4.7 (0.9) <i>b</i>	5.8 (1.0) <i>ab</i>	5.1 (1.0) <i>b</i>	9.0 (1.4) <i>a</i>	7.2 (1.0) <i>ab</i>
Shoot dry weight	7.6 (0.2) <i>a</i>	4.4 (1.1) <i>bc</i>	5.4 (1.4) <i>ab</i>	14.6 (0.3) <i>c</i>	7.2 (1.4) <i>a</i>	6.1 (1.0) <i>a</i>	8.2 (0.8) <i>a</i>	6.2 (1.0) <i>a</i>
Root dry weight	5.1 (0.6) <i>a</i>	3.4 (1.1) <i>ab</i>	2.3 (0.8) <i>bc</i>	0.8 (0.4) <i>c</i>	2.9 (1.0) <i>a</i>	4.1 (0.6) <i>a</i>	3.9 (0.6) <i>a</i>	3.5 (0.5) <i>a</i>
Total dry weight	12.7 (2.2) <i>a</i>	7.8 (1.4) <i>a</i>	7.7 (1.6) <i>a</i>	2.5 (1.7) <i>b</i>	10.1 (1.1) <i>a</i>	10.2 (0.9) <i>a</i>	11.2 (0.7) <i>a</i>	9.7 (0.9) <i>a</i>
Leaf tissue chlorophyll	3.2 (0.5) <i>a</i>	2.4 (0.3) <i>a</i>	2.2 (0.52) <i>a</i>	2.1 (0.6) <i>a</i>	3.1 (0.2) <i>a</i>	2.2 (0.3) <i>b</i>	3.5 (0.1) <i>a</i>	2.2 (0.1) <i>b</i>
SC	149 (28) <i>a</i>	207 (37) <i>a</i>	187 (38) <i>a</i>	189 (39) <i>a</i>	125 (20) <i>bc</i>	199 (29) <i>ab</i>	230 (36) <i>a</i>	93 (15) <i>c</i>
Root porosity	18.0 (8.2) <i>a</i>	26.3 (8.8) <i>a</i>	31.7 (3.3) <i>a</i>	22.4 (7.1) <i>a</i>	30.3 (5.7) <i>ab</i>	17.2 (13.1) <i>b</i>	34.7 (4.2) <i>a</i>	22.2 (4.9) <i>ab</i>
Species	<i>Sagittaria lancifolia</i>							
Height growth	33.3 (9.1) <i>a</i>	16.8 (4.6) <i>a</i>	41.3 (5.5) <i>a</i>	28.5 (9.8) <i>a</i>	20.7 (10.6) <i>ab</i>	11.8 (7.0) <i>ab</i>	14.3 (7.1) <i>b</i>	40.8 (2.3) <i>a</i>
SC	197 (12) <i>a</i>	133 (14) <i>b</i>	205 (17) <i>a</i>	202 (25) <i>a</i>	202 (25) <i>a</i>	213 (22) <i>a</i>	269 (41) <i>a</i>	213 (25) <i>a</i>

\*Different letters within soil types across columns represent significant differences ( $p < 0.05$ ) among treatments as determined by Duncan's Multiple Range Test using SAS system; see Table 1 for symbol description.

Key to treatment abbreviations: JNWF+, Jiffy Mix no waterlogging fertilized; JNWF–, Jiffy Mix no waterlogging no fertilizer; JWF+, Jiffy Mix waterlogged fertilized; JWF–, Jiffy Mix waterlogged no fertilizer; MNWF+, Metro Mix no waterlogging fertilized; MNWF–, Metro Mix no waterlogging no fertilizer; MWF+, Metro Mix waterlogged fertilized; MWF–, Metro Mix waterlogged no fertilizer.

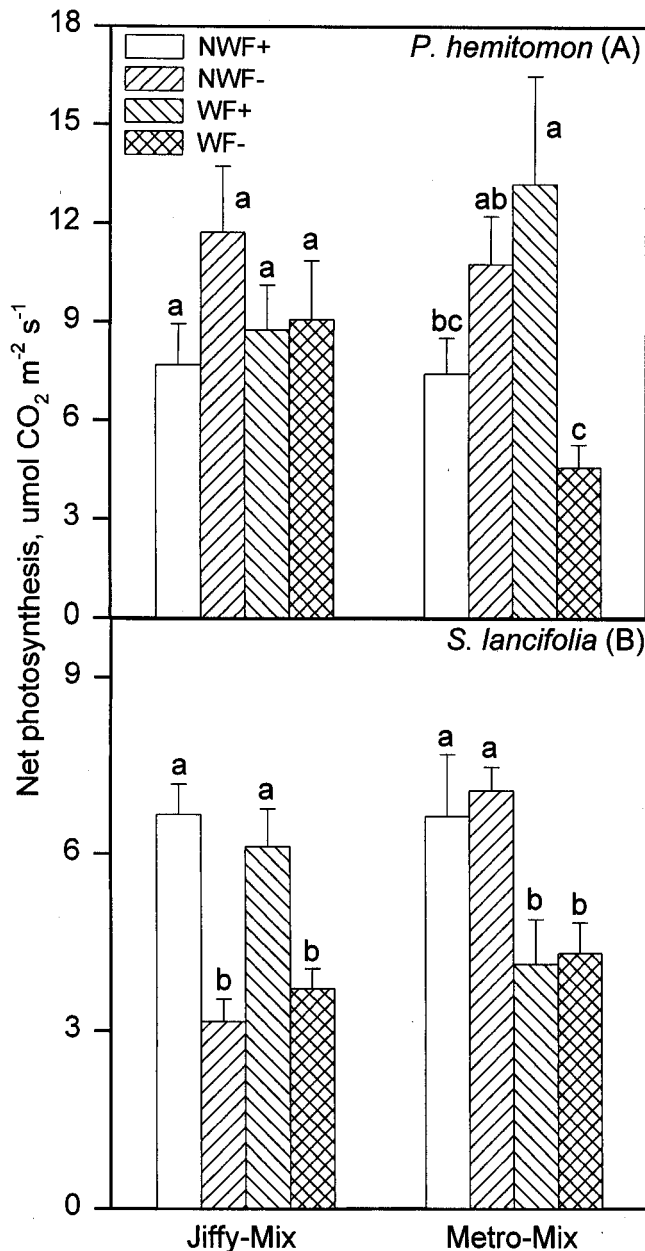


Figure 2. Responses of net photosynthesis (NP) in *Panicum hemitomon* (A) and *Sagittaria lancifolia* (B) to various soil mixture, waterlogging, and fertilizer. Values represent the mean obtained during Phase I of the experiment. Bars represent the mean  $\pm$  SE. Different letters represent significant differences ( $p < 0.05$ ) across treatments as determined by Duncan's Multiple Range Test using SAS system. Treatment abbreviation: JNWF+, Jiffy Mix no waterlogging fertilized; JNWF-, Jiffy Mix no waterlogging no fertilizer; JWF+, Jiffy Mix waterlogged fertilized; JWF-, Jiffy Mix waterlogged no fertilizer; MNWF+, Metro Mix no waterlogging fertilized; MNWF-, Metro Mix no waterlogging no fertilizer; MWF+, Metro Mix waterlogged fertilized; MWF-, Metro Mix waterlogged no fertilizer.

**Fertilization (MNWF+ and JNWF+).** *Panicum* grown in Metro Mix and fertilized during Phase I had enhanced SC and NP during Phase II as compared to control plants (MNWF-) (Table 3; Fig. 3A), indicating that the beneficial fertilizer effects on gas exchange functions had persisted. *Panicum* grown in Jiffy Mix had significant increases in the number of culms generated, shoot dry weight, root dry weight, and total dry weight compared to controls (JNWF-) (Table 3). Other plant parameters did not change significantly. Stomatal conductance of fertilized *Sagittaria* grown in Jiffy Mix was significantly greater compared to controls (JNWF-) during Phase II of the study (Table 3). There were no other significant differences between fertilized *Sagittaria* and controls (Table 3; Fig. 3B).

**Waterlogging (MWF- and JWF-).** *Panicum* grown in Metro Mix and waterlogged during Phase I had decreased NP during Phase II as compared to control plants (MNWF-) (Fig. 3A). For the two species, there were no other significant differences when previously waterlogged during Phase II, compared to their respective controls (JNWF-, MNWF-) (Table 3; Figs. 3A and B).

**Fertilization and Waterlogging (MWF+ and JWF+).** As in Phase I, previously flooded and fertilized *Panicum* in both soil types had a significantly higher number of new culms formed compared to controls (JNWF-, MNWF-) (Table 3). This finding indicated the benefit of the combined fertilizer and flooding for improving regeneration in *Panicum*. In addition, previously waterlogged and fertilized *Panicum* grown in Jiffy Mix had significant increases in shoot, root, and total dry biomass compared to controls (JNWF-) (Table 3). There were no significant differences in previously waterlogged and fertilized *Sagittaria* during Phase II compared to controls (JNWF-, MNWF-) (Table 3; Fig. 3).

The ANOVA for NP revealed significant two-way interactions between species and flooding ( $p = 0.044$ ) and species and fertilizer ( $p = 0.029$ ). There was also a significant interaction between species, soil, and fertilizer for NP ( $p = 0.001$ ), reflecting the differences in gas exchange responses to various treatments between the two species. For leaf chlorophyll concentration, there were significant two-way interactions between species and soil ( $p = 0.001$ ) and waterlogging and fertilizer ( $p = 0.007$ ). There was also a significant interaction among species, waterlogging, and fertilizer ( $p = 0.048$ ) for leaf chlorophyll concentration. For the number of new culms, there was a significant three-way interaction among soil, waterlogging, and fertilizer ( $p = 0.020$ ). Shoot biomass was significantly affected by a three-way interaction of species, waterlogging, and fertilizer ( $p = 0.021$ ), whereas for root biomass there was a significant interaction between species and soil type ( $p = 0.043$ ).

**Table 3.** Responses of height growth (final height – initial height, cm), number of culms per pot (shoot), shoot dry weight per pot (g), root dry weight per pot (g), total dry weight per pot (g), leaf tissue chlorophyll concentration (mg/g Fw), stomatal conductance (SC, mmol H<sub>2</sub>O/m<sup>2</sup> leaf/s), and root porosity (% airspace) for *Panicum hemitomon* and *Sagittaria lancifolia* at the conclusion of Phase II.\*

	Treatment							
	JNWF +	JNWF –	JWF +	JWF –	MNWF +	MNWF –	MWF +	MWF –
Species	<i>Panicum hemitomon</i>							
Height growth	8.7 (2.3) <i>a</i>	12.6 (3.6) <i>a</i>	11.4 (3.8) <i>a</i>	4.8 (3.0) <i>a</i>	18.6 (2.5) <i>a</i>	14.7 (3.4) <i>a</i>	21.0 (3.5) <i>a</i>	23.3 (4.7) <i>a</i>
Number of culms	13.7 (1.2) <i>a</i>	9.0 (1.2) <i>b</i>	16.3 (0.8) <i>a</i>	7.5 (0.9) <i>b</i>	11.2 (0.9) <i>b</i>	11.8 (1.4) <i>b</i>	18.0 (2.3) <i>a</i>	10.8 (1.7) <i>b</i>
Shoot dry weight	19.1 (2.9) <i>a</i>	11.5 (2.2) <i>b</i>	19.0 (2.0) <i>a</i>	5.4 (0.7) <i>b</i>	16.6 (5.1) <i>ab</i>	21.5 (3.4) <i>ab</i>	25.6 (5.4) <i>a</i>	6.0 (2.1) <i>b</i>
Root dry weight	15.0 (1.9) <i>a</i>	8.3 (1.7) <i>b</i>	16.8 (1.1) <i>a</i>	4.7 (0.4) <i>b</i>	16.3 (3.5) <i>ab</i>	15.6 (1.0) <i>ab</i>	22.5 (3.0) <i>a</i>	9.4 (2.4) <i>b</i>
Total dry weight	34.1 (4.7) <i>a</i>	19.8 (3.9) <i>b</i>	35.8 (2.9) <i>a</i>	10.1 (1.1) <i>b</i>	32.9 (8.4) <i>ab</i>	37.1 (4.1) <i>ab</i>	48.1 (4.4) <i>a</i>	15.4 (8.4) <i>b</i>
Leaf tissue chlorophyll	1.2 (0.1) <i>a</i>	1.2 (0.1) <i>a</i>	1.0 (0.1) <i>a</i>	1.3 (0.1) <i>a</i>	2.4 (0.3) <i>a</i>	1.5 (0.2) <i>a</i>	1.9 (0.4) <i>a</i>	2.5 (0.3) <i>a</i>
SC	61 (6) <i>a</i>	52 (5) <i>ab</i>	44 (3) <i>b</i>	41 (3) <i>b</i>	107 (9) <i>a</i>	60 (4) <i>bc</i>	78 (10) <i>b</i>	41 (6) <i>c</i>
Root porosity	37.9 (2.5) <i>a</i>	33.2 (3.4) <i>a</i>	36.7 (2.3) <i>a</i>	40.7 (3.3) <i>a</i>	27.1 (5.5) <i>a</i>	33.7 (4.4) <i>a</i>	25.5 (4.9) <i>a</i>	27.4 (3.5) <i>a</i>
Species	<i>Sagittaria lancifolia</i>							
Height growth	10.5 (7.0) <i>a</i>	20.0 (5.6) <i>a</i>	5.8 (2.9) <i>a</i>	11.5 (5.9) <i>a</i>	13.0 (3.2) <i>a</i>	9.7 (9.0) <i>a</i>	21.8 (4.0) <i>a</i>	5.8 (8.3) <i>a</i>
Shoot dry weight	13.3 (6.4) <i>a</i>	4.0 (2.5) <i>a</i>	14.7 (4.3) <i>a</i>	8.3 (4.3) <i>a</i>	13.9 (5.8) <i>a</i>	11.7 (3.7) <i>a</i>	5.9 (2.5) <i>a</i>	6.0 (2.0) <i>a</i>
Root dry weight	10.9 (5.2) <i>a</i>	3.7 (2.4) <i>a</i>	8.7 (3.6) <i>a</i>	2.0 (1.7) <i>a</i>	9.9 (2.5) <i>a</i>	8.4 (2.1) <i>ab</i>	2.9 (1.0) <i>b</i>	3.3 (1.0) <i>b</i>
Total dry weight	24.2 (11.5) <i>a</i>	7.7 (4.9) <i>a</i>	23.4 (7.0) <i>a</i>	10.3 (6.0) <i>a</i>	23.8 (8.2) <i>a</i>	20.0 (5.8) <i>a</i>	8.8 (3.4) <i>a</i>	9.3 (2.7) <i>a</i>
Leaf tissue chlorophyll	1.1 (0.01) <i>a</i>	0.9 (0.2) <i>a</i>	1.0 (0.1) <i>a</i>	1.0 (0.1) <i>a</i>	1.1 (0.2) <i>a</i>	1.0 (0.1) <i>a</i>	1.2 (0.2) <i>a</i>	1.1 (0.1) <i>a</i>
SC	198 (22) <i>a</i>	138 (18) <i>b</i>	144 (20) <i>b</i>	134 (17) <i>b</i>	193 (24) <i>b</i>	197 (29) <i>a</i>	163 (17) <i>a</i>	191 (32) <i>a</i>
Root porosity	56.7 (5.5) <i>a</i>	52.8 (3.1) <i>a</i>	56.5 (3.6) <i>a</i>	59.7 (2.7) <i>a</i>	56.2 (2.5) <i>a</i>	52.4 (2.9) <i>a</i>	49.7 (4.5) <i>a</i>	55.6 (1.7) <i>a</i>

\*Different letters within soil types across columns represent significant differences ( $p < 0.05$ ) between treatments as determined by Duncan's Multiple Range Test using SAS system; see Table 1 for symbol description.

Key to treatment abbreviations: JNWF+, Jiffy Mix no waterlogging fertilized; JNWF–, Jiffy Mix no waterlogging no fertilizer; JWF+, Jiffy Mix waterlogged fertilized; JWF–, Jiffy Mix waterlogged no fertilizer; MNWF+, Metro Mix no waterlogging fertilized; MNWF–, Metro Mix no waterlogging no fertilizer; MWF+, Metro Mix waterlogged fertilized; MWF–, Metro Mix waterlogged no fertilizer.

## Discussion

We tested the hypotheses that, in producing planting stocks for wetland mitigation projects, the probability of success (high survival rates and growth of transplanted stocks) can be improved through flood pre-conditioning and fertilizer pre-conditioning treatments in the nursery, and that the pre-conditioned plants are likely to grow better than non-hardened or non-pre-conditioned plants of the same species. Do plants subjected to flooding in the nursery have a better survival/growth rate during subsequent flooding than plants grown under non-flooded conditions? Our data do not support the hypothesis that flood pre-conditioning alone can significantly improve plant survival and growth under subsequent waterlogging conditions, at least for our study species. This finding was attributed to the nature of the study species: being highly flood-tolerant and possessing many capabilities that allow them to survive under reducing soil conditions in their natural habitats. However, the flood pre-conditioning in our study lasted a maximum of 42 days. Such a period may not have provided adequate time for plant response development to become detectable. Longer periods of flood pre-conditioning than those tested in the present study and repeated exposure to flooding may improve the plants' subsequent performance in the field. This hypothesis requires additional testing. However, longer periods may not represent realistic durations because nurseries have space and cost efficiency issues that determine how long seedlings can remain in the nursery for such pre-conditioning.

Another question asked concerned the potential for beneficial interaction between fertilization and flooding, and the relationship to subsequent plant performance. Is there any significant interaction between flooding and fertilization leading to production of vigorous, tolerant planting stocks? Our data showed that fertilization alone or in combination with flooding appears to enhance shoot and root production in *P. hemitomon* during the subsequent flooding period, but such effects were apparent in only one of the soils (i.e., Jiffy Mix) (Table 3). In contrast, flooding alone (without fertilizer) produced *Panicum* plants that appeared to remain somewhat susceptible, characterized by low photosynthetic rates, to subsequent flooding during Phase II as compared to fertilized plants. *S. lancifolia* plants subjected to fertilizer treatment alone did not produce significantly greater total dry weights compared to controls (NWF–) (Table 3). Thus, a combination of flood and fertilizer pre-conditioning in the nursery appears to be helpful to some species; the results are species specific.

For wetland mitigation efforts to be successful (i.e., produce high plant survival rates and vigorous plant growth) the transplanted stocks must be able to withstand the reducing soil conditions that result from soil saturation at mitigation sites. Wetland soils could become devoid of oxygen during the periods of saturation/inundation, with limited or excess nutrient supplies depending on the soil conditions. Soil waterlogging for 5 to 100% of the growing season is a dominant feature of most wetland ecosystems. Such conditions begin a chain of physicochemical reactions in soils leading to reductions in soil E<sub>H</sub> (Ponnamperuma 1984). The resultant ef-

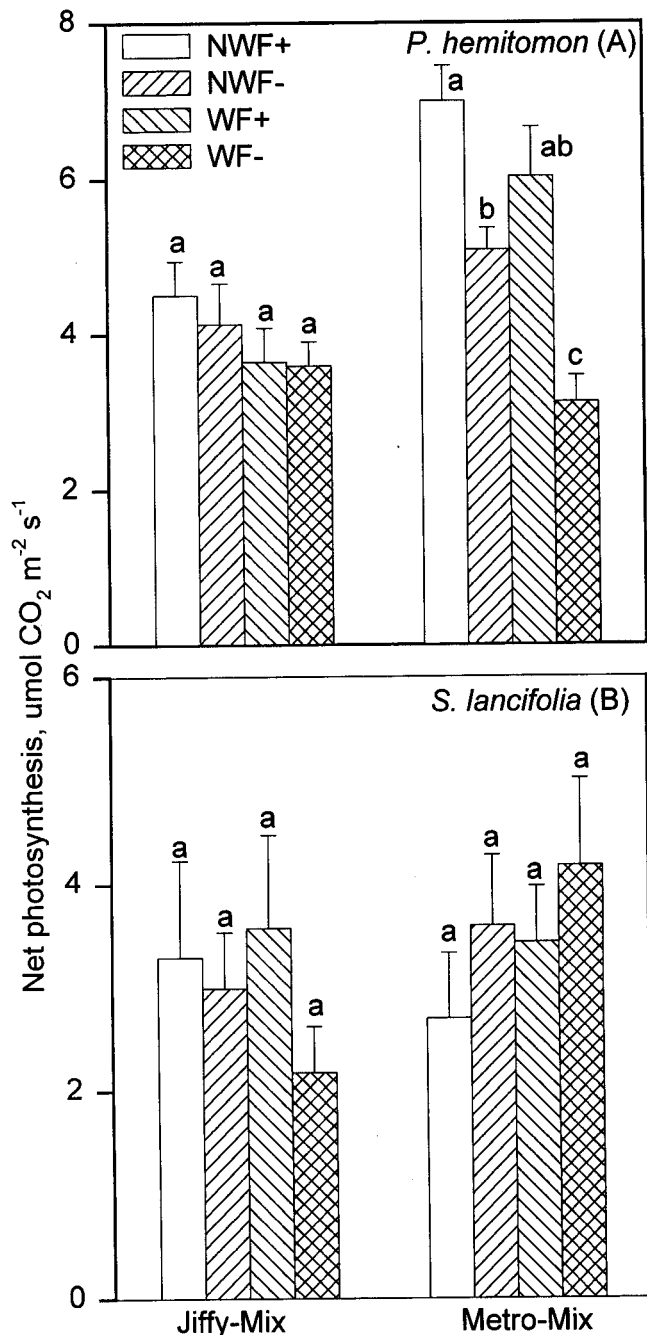


Figure 3. Responses of net photosynthesis in *Panicum hemitomon* (A) and *Sagittaria lancifolia* (B) to various soil mixtures and waterlogging. Prior treatments are the same as in Figure 2. Values represent the mean obtained during Phase II of the experiment. Bars represent the mean  $\pm$  SE. Different letters represent significant differences ( $p < 0.05$ ) across treatments as determined by Duncan's Multiple Range Test using SAS system. Note that during Phase II all plants were subjected to waterlogging only. Treatment abbreviation: JNWF+, Jiffy Mix no waterlogging fertilized; JNWF-, Jiffy Mix no waterlogging no fertilizer; JWF+, Jiffy Mix waterlogged fertilized; JWF-, Jiffy Mix waterlogged no fertilizer; MNWF+, Metro Mix no waterlogging fertilized; MNWF-, Metro Mix no waterlog-

fects on plants are a series of complex responses that may result in various symptoms ranging from mild responses to severe injury and death (Kozlowski 1984, 1997; Armstrong et al. 1994; Pezeshki 1994).

In most plants, including flood-tolerant wetland plants, root growth is restricted under anaerobic conditions where soils remain continuously reduced (Pezeshki 1991; Armstrong et al. 1994). In the present study, root growth of *Panicum* grown in Jiffy Mix was enhanced in response to prior fertilization (during Phase I), irrespective of flooding treatment. However, *Sagittaria* showed no significant patterns of root responses to the prior treatments (Table 3).

Among mechanisms developed to cope with oxygen-deficient environments are root morphological/anatomical responses, which enhance root porosity and facilitate root oxygenation. A significant increase in root porosity of wetland plants in response to root hypoxia has been reported for U.S. Gulf coastal vegetation (Pezeshki et al. 1993). There were no significant changes in root porosity in response to the treatments in either species at the conclusion of this study. Nevertheless, both species had high root porosity, averaging 55 and 33% in *Sagittaria* and *Panicum*, respectively. This finding was attributed to the fact that all plants were subjected to flooding during Phase II and, thus, aerenchyma tissue development was primarily in response to the flooding. Both species are capable of developing high root porosity in saturated soils and do not appear to need flood pre-conditioning in the nursery to achieve this.

Among the early responses of plants to soil flooding are plant gas exchange responses (Pezeshki 1994; Kozlowski 1997). In the present study, plant gas exchange functions showed distinct variations in response to flooding across treatments. Stomatal conductance and net photosynthetic responses to various treatments were different between the two species during Phase II, responding to soil and/or other pre-conditioning treatments applied during Phase I (Table 3). *Panicum* showed significantly greater photosynthesis in Metro Mix for those plants that were subjected to fertilization but no waterlogging (NWF+) as compared to other treatments. In contrast, none of the Phase I treatments appeared to have any influence on subsequent photosynthetic activity of *Sagittaria* plants that were subjected to continuous flooding during Phase II. Thus, it appears that pre-conditioning treatments had little impact on subsequent responses of the latter species to flooding.

Reduced sediment conditions in marshes and the concomitant root hypoxia can result in physiological stress, limiting root ability for active nutrient uptake or

ging no fertilizer; MWF+, Metro Mix waterlogged fertilized; MWF-, Metro Mix waterlogged no fertilizer.

creating nutrient imbalance. The nutrient status of plant tissue can, in turn, affect plant photosynthetic activity, growth, and productivity (DeLaune & Pezeshki 1988; Bandyopadhyay et al. 1993). Thus, the beneficial effects of fertilizer addition to soil medium in the nursery prior to transplanting stocks at a mitigation site are obvious. In the present study, the beneficial effects of fertilization (during Phase I) on subsequent plant gas exchange under flooding (Phase II) were apparent for *Panicum* grown in Metro Mix when comparing plants under NWF+ treatment versus plants under NWF- treatment in Phase II (Fig. 3A). The fertilizer benefit appears to be dependent on the absence of flooding (during Phase I). Additional support for such an argument is apparent when comparing the biomass production under the two treatments in both soils (Table 3). Although no clear biomass response patterns to fertilization could be concluded from the present data for *Sagittaria*, significant enhancement in gas exchange was noted in this species.

Our data indicate the potential for improvement in gas exchange, growth, and biomass of planting stocks produced for wetland mitigation by fertilization pre-conditioning in the nursery. However, the commercial soil medium appears to be an important factor. Additional research is needed to study responses of other wetland plant species to nursery pre-conditioning, including repeated waterlogging, different fertilizer regimes, and to test the subsequent performance of planting stocks in the field.

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